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(54) Title: USE OF DPPIV INHIBITORS AS DIURETIC AND ANTI-HYPERTENSIVE AGENTS

(57) Abstract: Dipeptidyl peptidase IV inhibitors such as Ile-Pro-Ile or a thiazolidide are used as diuretics and anti-hypertensive agents.

TITLE OF THE INVENTION

USE OF DPPIV INHIBITORS AS DIURETIC AND ANTI-HYPERTENSIVE AGENTS

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a continuation-in-part of U.S. application serial number 09/938,325, filed 23 August 2001, which claimed priority benefit of provisional application U.S. Ser. No. 60/227,400, filed 23 August 2000.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

The invention was made with partial government support under NIH grant DK-33793. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Field of the Invention. This invention relates to a new class of diuretics and antihypertensive agents and methods for their use.

Description of Related Art. Epidemiologic studies have clearly demonstrated that elevated blood pressure is correlated with an increased incidence of cardiovascular disease, including stroke, renal failure, congestive heart failure, and myocardial infarction. The prevalence of hypertension increases with age in all groups: blacks, whites, men, and women. Hypertension is an extremely common health problem in the geriatric population, afflicting approximately 65% of persons

in the 65- to 74-year-old group (Bennett, J.C., and Plum, F., eds., *Cecil's Textbook of Medicine*, 20th ed., W. B. Saunders Co., Philadelphia, 1996, p. 258; this reference and others cited herein are expressly incorporated in their entireties by reference). Blacks have a higher prevalence of hypertension than whites, and men have a higher overall prevalence than women (*ibid.*).

There is a great variety of drugs available for use in treating hypertension, including thiazide, loop, and potassium-sparing diuretics, β-adrenergic receptor blocking agents, calcium channel blockers, and angiotensin-converting enzyme inhibitors. Unfortunately, the adverse metabolic effects of some classes of antihypertensive drugs may increase coronary risk and offset the benefit of blood pressure reduction. Hence, there is considerable interest in developing new drugs, particularly those that act specifically on the regulation of renal sodium chloride (NaCl) excretion, which is responsible for maintaining NaCl balance and long-term control of blood pressure.

BRIEF SUMMARY OF THE INVENTION

It is the primary objective of this invention to provide a new class of diuretics that are agents useful for treating hypertension.

It is a further and more specific objective of the invention to provide therapeutic agents for inhibiting renal sodium absorption.

These and other objectives are achieved by the present invention, which provides methods for treating hypertensive patients by administering to them an effective amount of at least one dipeptidyl peptidase IV (abbreviated herein as "DPPIV") inhibitor. This is typically achieved by administering a pharmaceutical composition containing a DPPIV inhibitor. Exemplary inhibitors include peptides, acyl pyrrolidides and thiazolidides that are specific in their inhibition of DPPIV,

stable, and non-toxic, such as those described for the treatment of diabetes, particularly type 2 diabetes, including acyl pyrrolidides and isoleucine and valine thiazolidides (see, for example, Pederson, R.A., *et al., Diabetes 47*: 1253 (1998); Holst, J.J., and Deacon, C.F., *Diabetes 47*: 1663 (1998); Stocker, *et al., Diabetes, 50*: A522 (2001); Freyse, E.J., *et al., Diabetes 50*: A514 (2001); and Pospisilik, J.A., *et al., Diabetes 50*: A311 (2001)) and to suppress abortions in stressed animals (Hildebrandt, M., *et al., Immunology 53*: 449 (2001)). The use of the tripeptide Ile-Pro-Ile, also called diprotin A, is illustrated hereafter.

BRIEF DESCRIPTION OF THE INVENTION

This invention is based upon the finding that dipeptidyl peptidase IV inhibitors can be used to regulate renal sodium transport and have efficacy for the long term control of blood pressure.

In the practice of the invention, an effective amount of at least one dipeptidyl peptidase IV (also known as CD26) inhibitor is administered to a patient in need thereof in amounts effective to inhibit renal sodium resorption and/or act as a diuretic and anti-hypertensive agent. Though the invention is particularly useful in human therapies, as used herein, patients include both veterinary and medical patients. By "DPPIV inhibitor" is meant any inhibitor of dipeptidyl peptidase IV function, including traditional enzyme inhibitors, substrate analogues such as pseudosubstrates (including nonpeptide chemical pseudosubstrates), antibodies to DPPIV, enzymes that degrade it, and the like. Mixtures of inhibitors can also be employed, as well as inhibitors of enzyme synthesis or stability. In some embodiments of the invention, inhibitors are administered with at least one other compound that enhances the inhibitory effect and/or stabilizes the inhibitor in the formulation administered.

Administration of DPPIV inhibitors can be local or systemic. When

administered locally, at least one DPPIV inhibitor, preferably in association with a pharmaceutically acceptable carrier in which the inhibitor is dispersed or solubilized, is topically applied in effective amounts to the skin as a solution, lotion, cream, soap, and the like, or nasal mucosal and/or lung tissue using aerosols, inhalants, nasal drops, nasal sprays, and the like. Systemic administration of DPPIV inhibitors in other embodiments can be via any method known in the art such as, for example, oral administration of losenges, tablets, capsules, granules, or other edible compositions; subcutaneous, intravenous, intramuscular, or intradermal administration, *e.g.*, by sterile injections; parenteral administration of fluids and the like. Combinations of therapies may also be employed.

The amount of DPPIV inhibitor necessary to bring about the therapeutic treatment is not fixed *per se*, and necessarily is dependent upon the drug delivery to be enhanced, the particular inhibitor employed, the particular drug employed in combination with DPPIV inhibitor, adjunct compounds in the composition administered that enhance the inhibitory effect where present, the age, weight, and clinical condition of the patient to be treated, and the concentrations of these ingredients in the formulation put together in association with a pharmaceutically acceptable carrier. Generally the dose should be sufficient to enhance drug delivery without producing unacceptable toxicity to the patient.

As mentioned above, compositions of the invention are typically administered in admixture with a pharmaceutically acceptable carrier or vehicle. Administration is facilitated and, in some cases, additional therapeutic effects are provided by the carrier. When a carrier is employed, it is necessary that the carrier be inert in the sense of not bringing about a deactivation of inhibitor, and in the sense of not bringing about any adverse effect to the patient to whom it is administered.

Suitable carriers include any that will dissolve or disperse the active ingredients at concentrations of active ingredients most suitable for use in the

therapeutic treatment. Generally, even low concentrations of active ingredients in a carrier will be suitable, particularly where more frequent drug administration is required for enhancing drug therapy. It is desirable that compositions of the invention be formulated to contain amounts of inhibitor sufficient to provide blood pressure reduction of at least about 5%, preferably about 10% or higher, over what is observed in the absence of DPPIV inhibitor. Accordingly, carriers will be chosen which can solubilize or disperse the active ingredients at such concentrations. Examples of such carriers include both aqueous and nonaqueous carriers. In addition, pharmaceutical compositions or formulations may also include other carriers, adjuvants, stabilizers, preservatives, dispersing agents, and the like.

It should be understood that in addition to the ingredients particularly mentioned above, formulations of the invention may include other agents conventional in the art having regard to the type of formulation in question, for example, those suitable for nasal administration may include odors, for oral administration, flavoring agents, and for topical applications, emollients.

Useful DPPIV inhibitors include, but are not limited to, peptides, acyl pyrrolidides and thiazolidides previously described by Pederson, *et al.*, Holst and Deacon, Stocker, *et al.*, Freyse, *et al.*, Pospisilik, *et al.*, and Hildebrandt, *et al.*, cited above, as well as by ProBioDrug at www.probiodrug.de, who develop and market inhibitors for therapeutic use for the treatment of diabetes and Alzheimer's disease. Illustrated hereafter is a tripeptide inhibitor (Ile-Pro-Ile, also called diprotin A), but other peptides may be employed. Peptide inhibitors typically comprise three to five amino acids, at least one of which is a proline residue. Acyl pyrrolidides such as a thiazolidide, *e.g.*, isoleucine or valine thiazolidide, may be employed as an alternate or additional DPPIV inhibitor. P32/98 and P32/98 prodrugs described by Freyse, *et al.*, cited above, may also be used. It is an advantage of the invention that efficient and apparently nontoxic inhibitors exist, and the benefits of long term administration has been documented (see Holst and Deacon, Pospisilik, *et al.*, and Freyse, *et al.*, cited above).

EXAMPLES

The following examples are presented to further illustrate and explain the present invention and should not be taken as limiting in any regard.

The great majority of NaCl filtered by the kidney is reabsorbed in the first part of the nephron, the proximal tubule. In the proximal tubule, Na+-H+ exchanger isoform NHE3 is the most important pathway for mediating Na+ reabsorption.

In an attempt to identify proteins that assemble with NHE3, monoclonal antibodies (mAbs) against affinity-purified NHE3 protein complexes isolated from solubilized rabbit renal brush border membranes were generated. Hybridomas were selected based on ability to immunoprecipitate NHE3. One of these antibodies, 1D11, labeled a 110 kDa protein, but not monomeric NHE3 (80 kDa) in immunoblotting experiments. By immunofluorescence microscopy, 1D11 stained the brush border membrane of proximal tubule cells. To test if the "1D11 protein" is specifically associated with NHE3, immunoprecipitations were carried out using either the low speed (15,000xg for 10 min) or high speed (200,000xg for 1 hr) supernatants from Triton X-100 solubilized renal brush border membranes. MAb 1D11 co-precipitated NHE3 but not the microvillar protein villin from both low and high speed supernatants.

Having demonstrated that the 1D11 protein and NHE3 are truly associated, immunoaffinity chromatography was used to isolate the protein against which mAb 1D11 is directed. The 1D11 antibody was immobilized on Sepharose CL-4B beads, and then the 1D11 protein was purified from solubilized brush border membranes. N-terminal sequencing of the purified 1D11 protein identified it as dipeptidylpeptidase IV (DPPIV) (EC 3.4.14.5). Finally, to confirm that the 1D11 protein is DPPIV, a specific enzymatic assay for DPPIV was performed. It was

found that DPPIV enzymatic activity was virtually completely removed from solubilized rabbit brush border membranes passed through the same 1D11 affinity column, and was recovered in the eluted fractions.

Taken together, these experiments revealed an unexpected association of the brush border Na+-H+ exchanger NHE3 with dipeptidylpeptidase IV in the proximal tubule. This work describing the association of NHE3 with DPPIV has been published (Girardi, A.C.C., *et al., J. Am. Soc. Nephrol. 10*:4A, 1999, and Girardi, A.C.C., *et al., J. Biol. Chem. 276*: 46671 (2001)).

As an initial approach to examine the physiological role of the association of DPPIV with NHE3, diprotin A, a specific competitive inhibitor that binds to the active site of DPPIV, affects NHE3 activity in OKP cells was studied. OKP is a line of opossum proximal tubule cells that has transport properties very similar to the native proximal tubule and is therefore commonly used as an *in vitro* model for the mammalian proximal tubule.

The concentration dependence for diprotin A inhibition of DPPIV activity was first evaluated. A colorimetric enzyme assay using glycylproline p-nitroanilide tosylate as substrate showed that activity of DPPIV in OKP cells is completely inhibited by 1 mM diprotin A. ^{22}Na uptake assays were performed in 24 well plates in which OKP cells were pre-incubated for 20 minutes in a NH3/NH4+ buffer, pH 7.4, in the presence or absence of 1 mM diprotin A. NH_3/NH_4^+ buffer was then removed and cells were incubated with a NH_3/NH_4^+ free solution containing ^{22}Na and either vehicle or 1 mM diprotin A. It was confirmed that Na^+ influx measured under these conditions is ethylisopropyl-amiloride (EIPA)-sensitive and HOE-694 resistant, consistent with NHE3 activity. Diprotin A inhibited EIPA-sensitive Na/H exchange activity by 36.7 $\pm$ 5.3%. To test the specificity of this inhibition, activity of another brush border transport process, Cl^--formate exchange, measured as Cl^- gradient-stimulated, DIDS-sensitive uptake of 14C-formate was assayed. There was no significant effect of

diprotin A on Cl^--formate exchange activity.

It was concluded from these studies that the DPPIV inhibitor diprotin A decreases NHE3 activity in OKP cells. These results suggest that the association of DPPIV with NHE3 in oligomeric complexes may be involved in regulation of NHE3 activity. Moreover, these studies suggested that DPPIV inhibitors may be useful therapeutic agents for inhibiting renal Na+ reabsorption in the proximal tubule and therefore may be of use as diuretic and anti-hypertensive agents.

To further examine the possible role of DPPIV in modulating NHE3 activity, it was evaluated whether specific competitive inhibitors that bind to the active site of DPPIV affect NHE3 activity in the OKP line of proximal tubule cells. For this purpose isoleucine thiazolidide (sometimes referred to in the scientific literature as P32/98), a high affinity competitive inhibitor of DPPIV, was used as well as its inactive optical isomer (P34/98) as a negative control.

Na^+/H^+ exchange was assayed as ^{22}Na uptake into cells acid-loaded by the NH_4^+ prepulse technique. It was confirmed that Na influx measured under these conditions was S3226-sensitive and HOE-694 resistant, consistent with NHE3 activity. Addition of 10 μM P32/98 inhibited NHE3 activity by 46.7 $\pm$ 4.7%. In contrast, the inactive isomer P34/98 did not affect NHE3 activity. To test the specificity of this inhibition, the activity of another brush border transport process, Na/Pi cotransport, was also assayed. There was no significant effect of DPPIV inhibitors on Na/Pi cotransport activity in OKP cells.

These results demonstrated that a high affinity competitive inhibitor of the active site of DPPIV reduces NHE3 activity. Thus, these findings suggested that DPPIV, which physically associates with NHE3 in renal brush border membranes, has a tonic effect to stimulate NHE3 activity in proximal tubule cells. Accordingly, it DPPIV inhibitors can be used to inhibit renal tubular Na+ reabsorption, thereby achieving a diuretic and anti-hypertensive effect.

The above description is for the purpose of teaching the person of ordinary skill in the art how to practice the present invention, and it is not intended to detail all those obvious modifications and variations of it which will become apparent to the skilled worker upon reading the description. It is intended, however, that all such obvious modifications and variations be included within the scope of the present invention, which is defined by the following claims. The claims are intended to cover the claimed components and steps in any sequence which is effective to meet the objectives there intended, unless the context specifically indicates the contrary.

CLAIMS

1. A method for treating hypertension in a patient in need thereof comprising administering to the patient an effective amount of a dipeptidyl peptidase IV inhibitor.

2. A method for providing a patient in need thereof with a diuretic comprising administering to the patient an effective amount of a dipeptidyl peptidase IV inhibitor.

3. A method according to claims 1 or 2 wherein the inhibitor is a peptide.

4. A method according to claim 3 wherein the peptide comprises three to five amino acids.

5. A method according to claim 3 wherein the peptide has a proline residue.

6. A method according to claim 3 wherein the peptide comprises 3 amino acids.

7. A method according to claim 3 wherein the peptide is Ile-Pro-Ile.

8. A method according to claims 1 or 2 wherein the inhibitor is a non-peptide pseudosubstrate.

9. A method according to claims 1, 2, or 8 wherein the inhibitor is an acyl pyrrolidide.

10. A method according to claim 9 wherein the inhibitor is a thiazolidide.

11. A method according to claim 10 wherein the thiazolidide is isoleucine thiazolidide.

12. A method according to claim 10 wherein the thiazolidide is valine thiazolidide.

13. A method according to claims 1 or 2 wherein the inhibitor is an antibody.

14. A method according to any of the above claims wherein administration is oral.

15. A method according to any of the above claims wherein administration is subcutaneous.

16. A method according to any of the above claims wherein administration is intramuscular.

17. A method according to any of the above claims wherein administration is intravenous.

18. A pharmaceutical composition useful in the treatment of hypertension comprising a DPPIV inhibitor.

19. A pharmaceutical composition useful as a diuretic comprising a DPPIV inhibitor.

20. A pharmaceutical composition according to claims 18 or 19 wherein the DPPIV inhibitor is selected from the group consisting of a peptide or a thiazolidinide.